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Effects of olive and fish oil Ca soaps in ewe diets on milk fat and muscle and subcutaneous tissue fatty-acid profiles of suckling lambs

B. Gallardo[1], P. Gómez-Cortés[2], A. R. Mantecón[3], M. Juárez[2], T. Manso[1] and M. A. de la Fuente[2†]

[1]*ETS Ingenierías Agrarias, Universidad de Valladolid, 34004 Palencia, Spain;* [2]*Instituto de Investigación en Ciencias de la Alimentación (CSIC-UAM), 28049 Madrid, Spain;* [3]*Instituto de Ganadería de Montaña (CSIC-ULE), 24346 Grulleros, León, Spain*

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Enhancing healthy fatty acids (FAs) in ewe milk fat and suckling lamb tissues is an important objective in terms of improving the nutritional value of these foods for the consumer. The present study examined the effects of feeding-protected lipid supplements rich in unsaturated FAs on the lipid composition of ewe milk, and subsequently in the muscle and subcutaneous adipose tissues of lambs suckling such milk. Thirty-six pregnant Churra *ewes with their new-born lambs were assigned to one of three experimental diets (forage/concentrate ratio 50 : 50), each supplemented with either 3% Ca soap FAs of palm (Control), olive (OLI) or fish (FO) oil. The lambs were nourished exclusively by suckling for the whole experimental period. When the lambs reached 11 kg BW, they were slaughtered and samples were taken from the* Longissimus dorsi *and subcutaneous fat depots. Although milk production was not affected by lipid supplementation, the FO diet decreased fat content (*$P < 0.001$*), whereas the OLI milk FA profile resembled that of the Control diet. In contrast, although FO drastically diminished the contents of stearic and oleic acids (*$P < 0.001$*), all the saturated even-numbered carbon FAs from 6:0 to 14:0 increased (*$P < 0.05$*). FO also produced the highest levels of* c9,t11-18:2 *(2.21%) and n-3 FAs, 20:5 n-3 (0.58%), 22:5 n-3 (0.48%) and 22:6 n-3 (0.40%). The high levels of* trans-*11 18:1 (7.10%) obtained from the FO diet would suggest that Ca soaps only confer partial protection in the rumen. In contrast, the lack of significant differences in* trans-*10 18:1 levels (*$P > 0.05$*) and other* trans-*FAs between Control and FO treatments would indicate that FO treatment does not alter rumen biohydrogenation pathways under the assayed conditions. Changes in dam milk FA composition induced differences in the FA profiles of meat and fat depots of lambs, preferentially incorporated polyunsaturated FAs into the muscle rather than storing them in the adipose tissue. In the intramuscular fat of the FO treatment, all the n-3 FAs reached their highest concentrations: 0.97 (18:3 n-3), 2.72 (20:5 n-3), 2.21 (22:5 n-3) and 1.53% (22:6 n-3). In addition, not only did FO intramuscular fat have the most* cis-*9,* trans-*11 18:2 (1.66%) and* trans-*11 18:1 (3.75%), but also the lowest n-6/n-3 ratio (1.80) and saturated FA content were not affected. Therefore, FO exhibited the best FA profile from a nutritional point of view.*

Keywords: suckling lamb, fatty acid, milk, intramuscular fat, Ca soap

Implications

Altering the fatty-acid (FA) composition of ruminant-derived foods provides the means by which FA consumption in the human population can be aligned with public health policies. Fish oil Ca soap supplementation of ewe diets could be a useful strategy to improve the nutritional quality of their milk fat and consequently suckling ruminant fat depots. Although this can induce a decrease in fat content in the milk, an increase in n-3 FAs and CLA, together with the steady levels of saturated and *trans*-FA, would justify this approach to enhance the nutritional value of these ruminant products.

Introduction

In many Mediterranean areas, dairy sheep produce suckling lambs that are raised exclusively on maternal milk and slaughtered when they are between 30 and 45 days of age. This suckling lamb meat is popular and considered a high-quality food, although its fatty-acid (FA) profile does not

† E-mail: mafl@if.csic.es

adequately meet nutritional recommendations because of its high content of some saturated fatty acids (SFAs) and their potential hypercholesterolemic effects. Therefore, there is currently much interest in adding value to sucking lamb meat by reducing the SFA content and increasing the levels of some specific FAs, which are thought to be beneficial for human health, such as oleic acid, CLA and n-3 polyunsaturated fatty acids (PUFAs). Moreover, the proportion of some *trans*-FAs, which result from an incomplete biohydrogenation (BH) of PUFA in the rumen, should be limited owing to their detrimental effects on human health (Shingfield *et al*., 2013).

As the FA profile of suckling lamb meat tends to reflect the FA profile of their mother's milk, changing the FA composition of the dam's milk by dietary means could also change the FA profile of the suckling lamb's meat, as previously reported (Osorio *et al*., 2007). Several nutritional strategies have been studied to improve the FA profile of milk and suckling lamb meat, mostly by including in the ewe diet-unsaturated fats (plant oils, oilseeds, and marine and fish oils (FOs)) (Osorio *et al*., 2007; Manso *et al*., 2011).

Oleic acid is the major monounsaturated fatty acid (MUFA) in ruminant feeds, and some studies have reported increases in this FA in milk and lamb meat when olive oil (OLI) is added to the diet (Pérez Alba *et al*., 1996; Manso *et al*., 2011). Adding marine oils to dairy ewe diets has also proven to be an effective nutritional strategy for enhancing the milk content of some bioactive FAs, such as *cis*-9, *trans*-11 18:2 (RA), its precursor *trans*-11 18:1 (VA) and, to a lesser extent, n-3 FAs (Toral *et al*., 2010a and 2010b). Although the transfer efficiency of 20:5 n-3 and 22:6 n-3 to milk fat is low owing to a highly incomplete BH by the ruminal bacteria, some studies have shown that this transfer efficiency to milk fat is greater when oils are fed in a rumen-protected form (Kitessa *et al*., 2003). Conversely, FOs generally cause a shift in the rumen BH pathways, with increases in ruminal outflow of specific *trans*-FAs (e.g. *trans*-10 18:1), which leads to a reduction in milk fat synthesis in the mammary gland (Shingfield *et al*., 2006), and to milk production with a low-fat content, which could potentially reduce suckling lamb growth rates.

Feeding animals with FAs in the form of Ca soaps from different oils could prevent, even if only partially, BH of PUFA in the rumen and reduce the proportion of SFAs and *trans*-FAs in the milk and meat. In this way, the adverse effects on cell wall digestion and organic matter fermentation in the rumen, resulting from the inclusion of PUFA-free lipids, could be avoided (Doreau *et al*., 2012). The aim of the present study was to evaluate the effects of different Ca soaps of FAs (CSFAs) of OLI and FO in the diet of *Churra* ewes on their milk FA profiles and performance, and on the intramuscular and subcutaneous FA composition of their suckling lambs. Because specific studies on the subsequent transfer of FAs from milk to lamb meat are limited, this study has also focused on the transfer efficiency of healthy FAs, such as oleic acid, and n-3 PUFA. Calcium soap of palm oil was used as a control, because it is a saturated fat commonly used in sheep feeding (Gargouri *et al*., 2006).

Material and methods

Animals and experimental diets

Thirty-six pregnant *Churra* ewes (mean BW 60.88 ± 1.366 kg) were selected before lambing and fed on the same diet that they would receive during the experimental period, but with no fat added (forage/concentrate ratio was 50 : 50). The ewes were 3 to 5 years old, their parity ranged from 4 to 6 and they had been artificially inseminated. Two days after lambing, each ewe was assigned to one of three experimental diets (12 ewes per treatment) based on their milk production, age, initial BW and parity randomization. The 36 new-born lambs (18 male and 18 female), covered by the protected geographical indication *Lechazo de Castilla y León*, were housed with their respective mothers all day long and nourished exclusively by suckling for the whole experimental period (from birth until they reached ~ 11 kg BW).

All animal handling practices followed the Directive 2010/63/EU of the European Parliament and the Council on the Protection of Animals used for experimental and other scientific purposes. The three experimental diets, which consisted of a total mixed ration (TMR), were isoenergetic and isonitrogenous, but varied according to the type of supplemented CSFAs (Table 1): Control, with 3% of Ca soap of palm oil (Magnapac®; Norel Animal Nutrition, Madrid, Spain); OLI, with 3% of Ca soap of OLI (Olifat®; Anupal S.L. Zaragoza, Spain); and FO, with 3% of Ca soap of FO (Strata-g Lactation®; Virtus Nutrition LLC., Corcoran, USA). The FA profiles of fat supplements are supplied

Table 1 *Ingredients and chemical composition of the ewe experimental diets*

	Control	OLI	FO
Ingredients, as fed (%)			
Dehydrated alfalfa	39.38	39.38	39.38
Soybean meal	13.77	13.77	13.77
Corn grain	11.83	11.83	11.83
Oat grain	10.38	10.38	10.38
Barley grain	7.86	7.86	7.86
Beet pulp	7.86	7.86	7.86
Molasses	4.95	4.95	4.95
Magnapac[1]	3	0	0
Olifat[2]	0	3	0
Strata-g[3]	0	0	3
Vitamin–mineral premix	1.00	1.00	1.00
Chemical composition (% DM)			
DM (%)	88.87	88.65	88.77
Ash	9.07	9.35	9.56
NDF	28.34	27.70	26.71
ADF	17.56	17.09	16.24
CP	16.86	16.38	17.61
Ether extract	5.30	5.30	5.32

OLI = olive oil; FO, fish oil; DM, dry matter.
[1]Magnapac® = calcium soap of palm oil, fatty-acid composition (%): 14:0, 1.20; 16:0, 46.9, 18:0, 40.7; 18:1, 9.70; 18:2, < 0.1; 18:3, < 0.1; 20:5, < 0.1; 22:6, < 0.1.
[2]Olifat® = calcium soap of olive oil, fatty-acid composition (%): 14:0, 1.40; 16:0, 8.2, 18:0, 2.90; 18:1, 53.7; 18:2, 15.6; 18:3, 3.10; 20:5, 0.2; 22:6, < 0.1.
[3]Strata-g® = calcium soap of fish oil, fatty-acid composition (%): 14:0, 2.2; 16:0, 8.1, 18:1, 17.2; 18:2, 4.5; 18:3, 1.5; 20:5, 19.6; 22:6, 9.7.

in Table 1. The CSFA of palm oil was used as a control, because it is commonly used in sheep feeding.

Each ewe was individually fed and received a total of 34.1 g of TMR per kg BW (on average 2.1 kg dry matter per head per day) of the corresponding experimental diet, *plus* 210 g of barley straw per head per day and fresh water *ad libitum*. Each ewe consumed the whole amount of TMR supplied daily.

Samples of diets were taken once a week during the whole experimental period and their chemical composition was determined using the procedures described by the AOAC (2003). There were no statistical differences in chemical composition between experimental diets.

Milk sampling and composition

The ewes were milked once a day in a 2×24 low-line Casse system milking parlour, with 12 milking units and 2 milkers. The milking machines (Alfa-Laval Iberia, S.A., Madrid, Spain) were set to provide 180 pulsations per minute with a 50:50 ratio at a vacuum level of 36 kPa. Milk production was recorded once a week. For this, lambs were separated from their mothers, and ewes were milked twice a day before feeding. In addition, milk samples were taken in milk collection jars. One sub-sample of milk was kept at 4ºC until analysed for fat and protein, according to the International Dairy Federation (2000), using a MilkoScan-400 analyser (Foss Electric, Hillerød, Denmark). Aliquots from weeks 2 and 4 of the experimental period were stored at –80ºC for FA analysis.

Slaughter procedure and carcass sampling

Lambs were weighed twice a week until they reached the intended BW (~11 kg). Hot and cold carcasses were weighed and the dressing percentage was calculated as the ratio of cold carcass weight to slaughter live weight. Fat samples of the *Longissimus dorsi* muscle (dissected between the 6th and the 13th rib) and subcutaneous dorsal fat (dissected from the rump) were taken and frozen at –80ºC until FA composition determination.

FA analysis

Milk fat was extracted according to Luna *et al.* (2008). Intramuscular fat extraction was carried out using Bligh and Dyer (1959) method, and subcutaneous fat was extracted by fusion of individual samples at 60°C in forced air oven. Milk and meat FA composition was determined by gas chromatography after FA methyl ester formation (Luna *et al.*, 2008).

Statistical analysis

Average daily weight gain was estimated as the regression coefficient (slope) of live weight against time using the REG procedure of SAS. Data of milk yield and composition (FAs included) were analysed by repeated measurement analyses using the MIXED procedure, which included the fixed effects of the diet (D), time on diet (T) and their interactions ($D \times T$). The rest of the parameters were statistically analysed by one-way ANOVA using GLM (PROC GLM). All the procedures were from the SAS 9.2. package (SAS Institute Inc., Cary, NC, USA).

Results and discussion

Milk yield and composition

The average daily milk yield and composition are recorded in Table 2. Neither milk yield nor protein yield and content were significantly modified by the dietary treatments ($P > 0.10$). However, milk fat content and total solids were significantly affected by the type of CSFAs included in the diet. OLI and FO supplements significantly decreased ($P < 0.001$) milk fat content, reaching reductions of 17% and 35%, respectively, compared with Control milk. Changes in total solid content varied in accordance with the decreases reported for milk fat. FO Ca soap reduced the percentage of milk solids ($P < 0.01$).

Previous studies have shown increases in milk yield and reductions in milk protein when sheep diets are supplemented with fat or oil (Pulina *et al.*, 2006). These changes can probably be explained by the greater energy content of the lipid-supplemented diets compared with the non-supplemented ones, and the reduced amino-acid availability in the mammary gland. Because the amount of feed offered to the animals was the same, and the diets assayed in the present experiment were isoenergetic, isonitrogenous and supplemented with some type of CSFA, no changes

Table 2 *Effect of supplementation of calcium soaps of olive (OLI) or fish (FO) oil and duration of supplementation on ewe milk yield and milk composition*

	Experimental diets				*P*-value[1]		
	Control	OLI	FO	r.s.d.	*D*	*T*	*D* × *T*
Yield (g/day)							
Milk	1686	1908	1597	1122	ns	ns	ns
Fat	89.2[a]	85.6[a]	56.8[b]	43.7	*	ns	ns
Protein	73.6	87.2	74.3	37.1	ns	ns	ns
Total solids	274.6	280.5	232.6	88.9	ns	ns	ns
Composition (%)							
Fat	5.42[a]	4.48[b]	3.53[c]	2.25	***	ns	ns
Protein	4.42	4.56	4.64	0.64	ns	ns	ns
Total solids	15.30[a]	14.86[a]	14.11[b]	1.12	**	ns	ns

[a–c] Means within a row with different superscripts differ significantly.
[1]Probability of significant effects because of experimental diets (D), time on diet (T) and their interaction ($D \times T$).
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

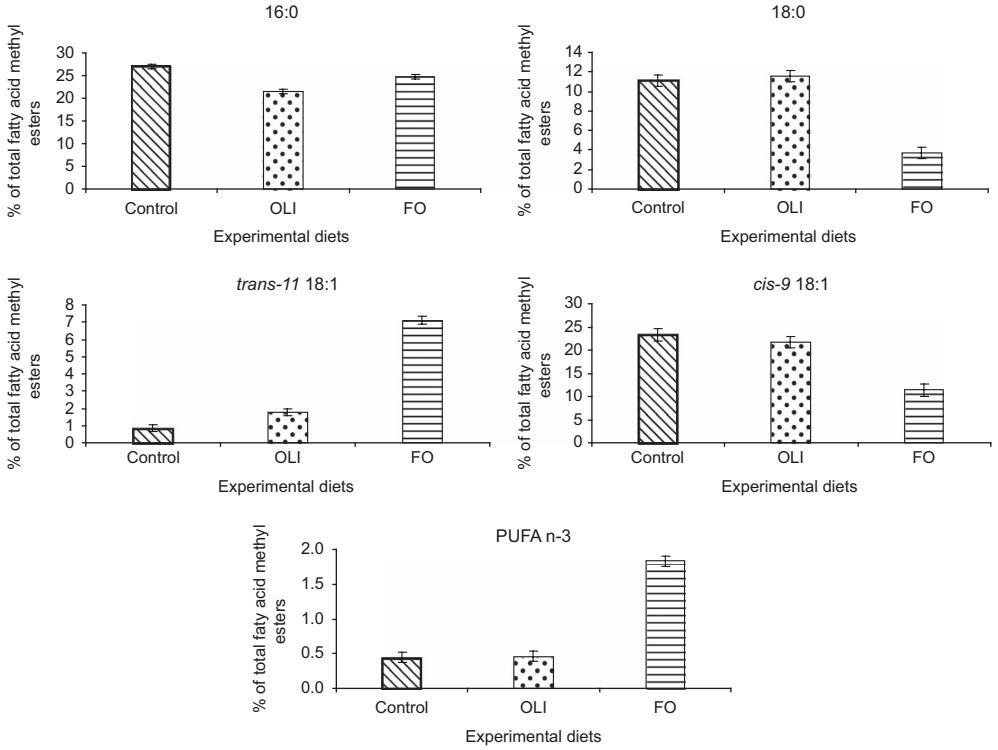

Figure 1 Mean values of 16:0, 18:0, *cis*-9 18:1, *trans*-11 18:1 and total n-3 fatty acids (18:3 + 20:5 + 22:5 + 22:6) in milk fat of ewes fed three experimental diets (forage/concentrate ratio 50:50), each supplemented with either 3% Ca soap fatty acids of palm (Control), olive (OLI) or fish (FO) oil.

in milk yield or in protein content were expected. Similar results were previously reported by Manso *et al.* (2011) when supplementation with different oils was compared using isoenergetic experimental rations. Concerning the decrease in milk fat yield and content observed in the FO treatment, the dietary FA profile as well as the rumen and mammary metabolism of these compounds may have played a dominant role, as is more extensively discussed below.

Milk FA composition

Table 3 shows the FA profile of milk fat from ewes fed with the different experimental diets. There were no differences ($P > 0.10$) in the percentages of total SFAs between treatments, whereas the lowest concentration of MUFAs ($P < 0.01$) and the highest concentration of PUFAs ($P < 0.001$) were observed in FO milk. Although CSFAs of OLI supplementation did not change ($P > 0.10$) the average percentage of some SFAs, such as 12:0, 14:0 and 18:0, it did decrease ($P < 0.001$) the 16:0 proportion compared with Control treatment (Figure 1). Milk from ewes supplemented with FO displayed the highest concentrations of even-numbered carbon SFAs from 6:0 to 14:0 and the lowest percentages for 18:0 ($P < 0.001$) (Figure 1).

The differences in MUFA concentrations were mainly due to changes in the *cis*-9 18:1 level, which was significantly lower in FO milk than in Control and OLI milk ($P < 0.001$). Supplementation with CSFAs of OLI led to a remarkable increase in a variety of *trans*-18:1 isomers ($P < 0.001$) in milk. FO ewes produced the milk with the highest concentration of VA, whereas Control ewes showed the lowest values ($P < 0.001$) (Figure 1). In contrast, milk from ewes supplemented with OLI and FO displayed the lowest concentrations of 18:2 n-6 ($P < 0.001$). The *cis*-9, *trans*-11 18:2 content in milk from ewes in the OLI group was significantly higher than Control but lower than FO ($P < 0.001$). In addition, FO milk presented the greatest concentration of 18:3 n-3 acid as well as other n-3 PUFAs such as 20:5 n-3, 22:5 n-3 and 22:6 n-3 ($P < 0.001$).

The highest content of 16:0 in Control milk fat can be attributed to the considerable amount of this FA in palm oil, the major FA (Table 1), and to the fact that 16:0 would not suffer changes in the digestive tract. Although Olifat® had the highest level of *cis*-9 18:1 in its CSFA, the content of this FA in OLI milk fat did not differ from that of Control milk fat (Figure 1). These results could be explained primarily by the abundance of 18:0 in the Control supplement (Table 1), as this FA is partly converted into *cis*-9 18:1 in the mammary gland via Δ-9 desaturase (Bichi *et al.*, 2012). In contrast, *cis*-9 18:1 from Olifat® would be susceptible to isomerization in rumen and involve the formation of several isomer *trans*-monoenes (Mosley *et al.*, 2002; Gómez-Cortés *et al.*, 2008). This fact would account for the significant increases in these isomers, mainly *trans*-10 18:1 and *trans*-11 18:1, in OLI milk fat (Table 3). Part of the VA in OLI milk fat could also be derived from the partial BH of 18:2 n-6 acid present in Olifat® but lacking in the fat supplement of the Control diet (Table 1).

The high content of *trans*-11 18:1 in FO milk (7.1%) confirms that calcium soap protection of fat is incomplete (Jenkins and Bridges, 2007; Shingfield *et al.*, 2013). VA accumulation in milk fat reflects the inhibitory action of n-3 PUFAs present in

Table 3 *Effect of supplementation of calcium soaps of olive (OLI) or fish (FO) oil and duration of supplementation (T) on ewe milk fatty-acid profiles (% of total fatty acid methyl esters)*

	Experimental diets				*P*-value[1]		
	Control	OLI	FO	r.s.d.	*D*	*T*	*D* × *T*
SFA							
4:0	4.54[a]	4.42[a]	3.72[b]	0.73	***	ns	ns
6:0	2.87[b]	3.20[ab]	3.43[a]	0.92	*	†	ns
8:0	2.36[c]	2.79[b]	3.43[a]	1.12	***	*	ns
10:0	6.03[c]	7.24[b]	9.34[a]	3.47	***	*	ns
12:0	3.18[b]	3.77[b]	5.02[a]	1.88	***	*	ns
13:0	0.09[b]	0.10[b]	0.16[a]	0.07	***	*	ns
14:0	7.47[b]	8.37[b]	10.03[a]	2.32	***	**	ns
iso-15:0	0.17[b]	0.18[b]	0.23[a]	0.05	***	ns	ns
anteiso-15:0	0.26[b]	0.29[b]	0.33[a]	0.09	**	ns	ns
15:0	0.63[b]	0.69[b]	0.87[a]	0.18	***	ns	ns
iso-16:0	0.19	0.19	0.20	0.05	ns	ns	†
16:0	27.10[a]	21.54[c]	24.75[b]	3.54	***	*	ns
17:0	0.61	0.58	0.66	0.29	ns	**	ns
iso-18:0	0.08[a]	0.06[b]	0.07[b]	0.04	†	**	ns
18:0	11.09[a]	11.57[a]	3.74[b]	3.97	***	**	ns
20:0	0.18[b]	0.22[a]	0.13[c]	0.05	***	ns	ns
Other SFA	0.27[b]	0.36[a]	0.34[a]	0.02	***	ns	ns
MUFA							
cis-9 10:1	0.19[b]	0.23[b]	0.31[a]	0.14	***	**	ns
cis-9 15:1	0.06[b]	0.07[b]	0.11[a]	0.02	***	†	ns
trans-9 16:1 + iso-17:0	0.32[b]	0.35[b]	0.75[a]	0.11	***	†	ns
cis-7 16:1	0.27[b]	0.26[b]	0.44[a]	0.09	***	ns	ns
cis-9 16:1 + anteiso-17:0	0.99[b]	0.91[b]	1.77[a]	0.32	***	ns	ns
cis-9 17:1	0.22	0.20	0.18	0.15	ns	*	ns
trans-4 18:1	0.03[b]	0.06[a]	0.02[b]	0.02	***	ns	ns
trans 5 18:1	0.03[b]	0.06[a]	0.03[b]	0.02	***	ns	ns
trans-6 + *trans*-7 + *trans*-8 18:1	0.32[b]	0.70[a]	0.23[c]	0.22	***	ns	ns
trans-9 18:1	0.26[c]	0.53[a]	0.36[b]	0.17	***	ns	ns
trans-10 18:1	0.39[b]	1.21[a]	0.78[ab]	1.42	*	ns	ns
trans-11 18:1	0.85[c]	1.77[b]	7.10[a]	1.56	***	ns	ns
trans-12 18:1	0.32[b]	0.56[a]	0.49[a]	0.23	***	ns	ns
cis-9 18:1	23.28[a]	21.74[a]	11.39[b]	9.03	***	*	ns
trans-15 + *cis*-11 18:1	0.50[c]	0.62[b]	1.02[a]	0.19	***	ns	ns
cis-12 18:1	0.21[b]	0.25[a]	0.08[c]	0.09	***	ns	ns
cis-13 18:1	0.04[b]	0.06[a]	0.05[b]	0.01	***	*	ns
trans-16 + *cis*-14 18:1	0.30[b]	0.37[a]	0.09[c]	0.11	***	ns	ns
cis-15 18:1	0.05[b]	0.08[a]	0.08[a]	0.02	***	ns	ns
cis-16 18:1	0.06	0.07	0.07	0.03	ns	*	ns
cis-11 20:1	0.05[c]	0.07[b]	0.20[a]	0.05	***	ns	ns
Other MUFA	0.12[b]	0.14[ab]	0.16[a]	0.02	*	**	ns
Non-conjugated 18:2							
trans-11, *trans*-15 18:2	0.01[b]	0.02[b]	0.07[a]	0.02	***	ns	ns
trans-11, *cis*-15 18:2	0.03[b]	0.09[b]	0.56[a]	0.23	***	ns	ns
trans-8, *cis*-12 + *cis*-9, *trans*-13 18:2	0.08[b]	0.11[a]	0.05[c]	0.04	***	ns	ns
18:2 n-6	2.40[a]	1.86[b]	1.83[b]	0.42	***	ns	ns
Other non-conjugated 18:2	0.38[b]	0.46[a]	0.29[c]	0.07	***	†	ns
Conjugated 18:2							
cis-9, *trans*-11 18:2	0.37[c]	0.68[b]	2.21[a]	0.56	***	*	ns
trans-10, *cis*-12 18:2	0.01	0.01	0.01	0.00	ns	ns	ns
Other conjugated 18:2	0.05[b]	0.06[b]	0.11[a]	0.01	***	ns	ns
Other PUFA							
18:3 n-6	0.04	0.04	0.03	0.03	ns	†	ns
18:3 n-3	0.31[b]	0.29[b]	0.38[a]	0.08	***	†	ns
20:4 n-6	0.12[b]	0.12[b]	0.15[a]	0.06	*	ns	ns

Table 3: (*Continued*)

	Experimental diets				*P*-value[1]		
	Control	OLI	FO	r.s.d.	*D*	*T*	*D* × *T*
20:5 n-3	0.03[b]	0.04[b]	0.58[a]	0.25	***	ns	ns
22:5 n-3	0.07[b]	0.08[b]	0.48[a]	0.14	***	ns	ns
22:6 n-3	0.03[b]	0.04[b]	0.40[a]	0.15	***	ns	ns
Other PUFA	0.07[b]	0.08[b]	0.10[a]	0.01	**	ns	ns
SFA	67.11	65.55	66.45	8.34	ns	*	ns
trans-MUFA	2.19[c]	4.88[b]	9.02[a]	1.64	***	ns	ns
MUFA	27.33[a]	28.77[a]	22.89[b]	5.76	**	**	ns
PUFA	3.93[b]	3.89[b]	7.18[a]	0.71	***	ns	ns
Total CLA	0.43[c]	0.75[b]	2.33[a]	0.57	***	*	ns
BCFA	0.73[b]	0.77[b]	0.89[a]	0.12	***	ns	ns
SCFA	15.99[b]	17.87[b]	20.23[a]	3.83	***	*	ns
MCFA	41.79[b]	37.75[c]	45.77[a]	4.33	***	**	ns
LCFA	42.09[a]	44.05[a]	33.34[b]	7.75	***	**	ns
Ratios[2]							
14:1 desaturase index[2]	0.01[a]	0.01[a]	0.01[b]	0.00	†	*	ns
18:1 desaturase index[2]	0.70[b]	0.71[b]	0.86[a]	0.07	***	ns	ns
CLA desaturase index[2]	2.03[b]	1.91[b]	2.40[a]	0.86	*	**	ns
n-6/n-3	5.94[a]	4.65[b]	1.31[c]	0.89	***	ns	ns

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; BCFA = branched-chain fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids.
[a–c] Means within a row with different superscripts differ significantly.
[1]Probability of significant effects because of experimental diets (*D*), time on diet (*T*) and their interaction (*D* × *T*).
[2]14:1 desaturase index = *cis*-9 14:1/(14:0 + *cis*-9 14:1); 18:1 desaturase index = *cis*-9 18:1/(18:0 + *cis*-9 18:1); CLA desaturase índex = *cis*-9, *trans*-11 18:2/(*cis*-9, *trans*-11 18:2 + *trans*-11 18:1).
†$P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

marine oil, mainly 22:6 n-3, on the final BH step of 18 carbon unsaturated FAs (AbuGhazaleh and Jenkins, 2004). The strong correlations determined between VA and 20:5 n-3 ($r = 0.85$, $P < 0.001$), 22:5 n-3 ($r = 0.90$, $P < 0.001$), 22:6 n-3 ($r = 0.88$, $P < 0.001$) and total PUFA n-3 ($r = 0.88$, $P < 0.001$) would support this statement. Moreover, VA accumulation in FO milk could explain the drastic reduction of 18:0 in this treatment (3.74 *v.* 11.09 *v.* 11.57 for FO, Control and OLI, respectively). Furthermore, this mechanism could also be indirectly responsible for the low percentage of *cis*-9 18:1 observed in FO milk. The reduction of 18:0 in ruminal outflow would therefore result in a lower contribution of 18:0 to the mammary gland in FO ewes, which in turn would limit the endogenous synthesis of *cis*-9 18:1 via Δ-9 desaturase, despite the increase in enzymatic activity in FO ewes (Table 3).

It is remarkable that, under the experimental conditions assayed, the *trans*-10 18:1 content of milk fat from ewes supplemented with Strata-g® did not differ from the other treatments (Table 3). The increase in *trans*-10 18:1 levels in milk fat has frequently been associated with diets supplemented with linoleic-rich fats and marine lipids, where a shift in linoleic-acid ruminal BH pathways because of disturbances in the rumen environment can occur (Shingfield *et al*., 2006; Or-Rashid *et al*., 2008). Furthermore, the low content of *trans*-10 18:1 in FO milk may be related to the forage/concentrate ratio (50:50) of the basal diet. On low-forage diets or high-concentrate diets containing plant oils or marine lipids, *trans*-10 18:1 often replaces VA as the major *trans*-MUFA in milk fat (Shingfield *et al*., 2013). The degree of protection offered by calcium soaps might help maintain the rumen environment unaltered and thus prevent changes in the ruminal BH pathways.

The increases in RA content achieved with OLI and especially with FO (Table 3) treatment correlated closely with the observed increases in *trans*-11 18:1 milk fat content ($r = 0.97$; $P < 0.001$). As is the case with other ruminants, this was probably owing to the fact that the RA content in ewe milk fat originates mainly from endogenous synthesis of the *trans*-11 18:1 produced in the rumen via Δ-9 desaturase in the mammary gland (Bichi *et al*., 2012). In contrast, the very low concentrations of other CLA isomers detected in the current experiment (Table 3) were the result of dietary PUFA conversion in the rumen.

The inclusion of FO in the ewe diet produced significant increases ($P < 0.001$) in n-3 PUFA content in milk fat (Figure 1), according to previous research in ovine milk (Kitessa *et al*. 2003; Toral *et al*., 2010a). Nevertheless, the apparent transfer of these dietary FAs, particularly 22:5 n-3 and 22:6 n-3, into milk was limited and represented an average transfer efficiency of 4% and 5%, respectively. The 22:5 n-3 and 22:6 n-3 levels observed in FO treatment can be attributed to their ruminal BH, despite being supplemented in a protected form. The BH of these PUFAs n-3 can be influenced by the concentration of the FO in the diet (Ashes *et al*., 1992). Results from a dose–response experiment with FO (Gulati *et al*., 1999) showed a greater capacity of sheep

rumen microorganisms to hydrogenate the 20:5 n-3 and 22:6 n-3 when the concentration of FO was < 1 mg/ml of rumen fluid. At this level, the production of *trans* 18:1 FA was maximum, but at higher concentrations of FO these isomers declined, indicating a potential rumen BH inhibition. Even in the absence of BH in the rumen, the potential to increase 22:5 n-3 and 22:6 n-3 is usually extremely limited. The lower transfer efficiency from the small intestine into milk for these n-3 FAs is thought to arise from the preferential incorporation of absorbed 22:5 n-3 and 22:6 n-3 into plasma phospholipids and cholesterol esters, rather than triacylglycerides of circulating chylomicrons (Kitessa *et al*., 2001; Shingfield *et al*., 2013). This fact would favour an uptake of only low proportions of these n-3 FAs into the mammary gland.

In contrast to OLI treatment, supplementation with CSFAs of FO reduced the oleic acid to approximately half the Control value and increased the 12:0, 14:0 and *trans*-MUFA content, which might not seem to be positive from a nutritional perspective. However, most *trans*-MUFA increases were linked to increases in *trans*-11 18:1, the physiological precursor of *cis-9*, *trans*-11 18:2 in tissue. Although the transfer of n-3 PUFA from diet into milk fat was limited, the n-6/n-3 ratio (Table 3) decreased 4.5- and 1.2-fold with FO and OLI diets, respectively, compared with the Control diet. The low n-6/n-3 ratio observed in FO milk would be positive from a nutritional point of view owing to its potential benefits for human health (Simopoulos, 2008) and its effect on the fat composition of suckling lamb meat, as explained below.

Milk fat depression (MFD)

The fall in milk fat content shown in Table 2 for the FO diet could be attributed to different causes. For instance, it has been proposed that in ewe, *trans*-10, *cis*-12 18:2 could be a milk fat synthesis inhibitor (Sinclair *et al*., 2007; Hussein *et al*., 2013). However, its percentages in the present study were negligible and no significant differences between treatments were detected (Table 3). Other FAs such as *trans*-10 18:1 (Shingfield *et al*., 2009) or *trans*-9, *cis*-11 18:2 (Perfield *et al*., 2007) could also be involved in MFD, but their amounts in all diets were low (Table 3), and therefore their roles as milk fat synthesis inhibitors could not be justified. Indeed, there was no significant correlation ($P > 0.05$) between changes in these FAs with diet and milk fat yield or content, and thus mechanisms other than direct inhibition had to be involved.

In this regard, it has been argued that the considerable impact of the maintenance of milk fat fluidity on milk fat secretion (Shingfield *et al*., 2006; Gama *et al*., 2008; Toral *et al*., 2010b) related to the incorporation of *cis*-9 18:1 and short-chain FAs (4:0 to 10:0) into triacylglycerides is the principal means of assuring milk fat liquidity at body temperature. Marine lipid inhibition of *trans*-18:1 ruminal saturation, thereby reducing the availability of 18:0 for endogenous mammary synthesis of oleic acid and simultaneously increasing *trans*-11 18:1 level (with a higher melting point than its equivalent *cis*-isomer) could reduce milk fat fluidity in FO treatment and would detrimentally affect milk fat maintenance. The intensified activity of Δ-9 desaturase in the mammary gland (Table 3) to generate *cis*-9 18:1 and *cis*-9, *trans*-11 18:2, as observed in the FO diet, could be thought as an adaptation to try to maintain and regulate milk fat fluidity for efficient secretion.

The FO treatment increased the milk content of FAs with < 16 carbons (i.e. synthesized *de novo* in the mammary gland), suggesting that there are other mechanisms by which the mammary gland tries to adapt to an altered supply of FAs. Thus, when CSFAs of FO were added to the diet, reduced oleic acid in the milk fat could be partially alleviated by an increase (Table 3) in the secretion of short- and medium-chain SFAs with low melting points. The aforementioned action would be further facilitated by the very low presence of *trans* isomers (*trans*-10 18:1, *trans*-10, *cis*-12 18:2 and *trans*-9, *cis*-11 18:2), generally associated with the inhibition of lipogenesis in the mammary gland.

Lamb performance

Significant differences ($P > 0.05$) were not observed in birth BW (4.01, 4.32 and 4.59 kg for Control, OLI and FO, respectively). The type of CSFAs added to ewe diets did not induce significant differences ($P > 0.05$) in growth (249, 232 and 225 g/animal per day for Control, OLI and FO, respectively), slaughter weight (10.8, 10.3 and 10.5 kg for Control, OLI and FO, respectively) and dressing percentage values (54.1%, 52.2% and 52.5% for Control, OLI and FO, respectively) between lambs of the different treatments, according to previous research (Casals *et al*., 2006; Awawdeh *et al*., 2009). Milk yield and composition are the principal factors responsible for suckling lamb growth. According to Gargouri *et al*. (2006), differences in protein intake are decisive in the growth of suckling lambs. Therefore, as the lambs in the current experiment were fed exclusively maternal milk, there would be no significant differences in milk protein yield and content. Furthermore, the lack of difference in weight and dressing percentages between lambs of the different treatments could be explained by the fact that the milk yield did not limit lamb growth.

Intramuscular and subcutaneous FA composition

No significant differences ($P > 0.05$) were found in intramuscular fat content (1.35, 1.19 and 1.19 g/100 g muscle for Control, OLI and FO treatment, respectively). Tables 4 and 5 show the FA composition of suckling lamb intramuscular and subcutaneous fats. There were no differences between treatment results for total SFAs in both fat depots of suckling lambs ($P > 0.10$) and dams' milk (Table 3). Following the same trend, intramuscular fat presented the highest MUFA content in Control and OLI treatments and the lowest in the FO lambs ($P < 0.01$). The FO lambs also had the highest proportion of PUFAs ($P < 0.05$) in their intramuscular fat consistent with the higher levels in FO ewe milk. Conversely, no differences between treatments ($P > 0.10$) were found in the MUFA and PUFA content of subcutaneous fat.

Regarding individual FA content, in general terms the observed differences reflected those found in the mother's milk, with the exception of the extremely low content of SFAs

Table 4 *Effect of milk from ewes receiving diets supplemented with Ca soap fatty-acid olive (OLI) and fish (FO) oil on fatty-acid composition (g/100 g of total fatty acid methyl esters) of intramuscular fat from suckling lambs*

	Control	OLI	FO	r.s.d.	*P*-value
SFA					
10:0	0.27^{ab}	0.23^{b}	0.36^{a}	0.11	†
12:0	0.37	0.40	0.60	0.33	ns
iso-14:0	0.28^{b}	0.31^{b}	0.52^{a}	0.10	***
14:0	4.23	3.84	4.78	1.37	ns
iso-15:0	0.09^{b}	0.08^{b}	0.11^{a}	0.02	**
anteiso-15:0	0.10^{b}	0.11^{b}	0.14^{a}	0.03	*
15:0	0.26^{b}	0.28^{b}	0.38^{a}	0.08	*
iso-16:0	0.13	0.12	0.13	0.03	ns
16:0	22.07^{a}	18.83^{b}	22.15^{a}	2.83	*
17:0	0.66	0.67	0.75	0.14	ns
iso-18:0	0.12	0.11	0.11	0.03	ns
18:0	12.69^{a}	12.62^{a}	10.16^{b}	1.85	*
19:0	0.11	0.11	0.13	0.04	ns
20:0	0.13	0.12	0.16	0.05	ns
22:0	0.39^{b}	0.44^{b}	0.57^{a}	0.12	*
23:0	0.24^{b}	0.24^{b}	0.42^{a}	0.11	**
Other SFA	0.10	0.11	0.13	0.04	ns
MUFA					
cis-9 14:1	0.18	0.16	0.20	0.05	ns
trans-9 16:1 + iso-17:0	0.43^{b}	0.50^{b}	0.88^{a}	0.13	***
cis-9 16:1 + anteiso-17:0	2.32^{ab}	1.97^{b}	2.49^{a}	0.44	†
cis-9 17:1	0.40	0.41	0.41	0.11	ns
trans-6 + *trans*-7 + *trans*-8 18:1	0.20^{b}	0.30^{a}	0.17^{b}	0.08	**
trans-9 18:1	0.22^{b}	0.33^{a}	0.27^{ab}	0.09	†
trans-10 18:1	0.27	0.56	0.36	0.44	ns
trans-11 18:1	0.57^{b}	1.27^{b}	3.75^{a}	0.95	***
trans-12 18:1	0.29^{b}	0.43^{a}	0.47^{a}	0.13	*
cis-9 18:1	31.95^{a}	32.22^{a}	20.71^{b}	5.32	***
trans-15 + *cis*-11 18:1	1.35^{b}	1.65^{b}	2.31^{a}	0.51	**
cis-12 18:1	0.49	0.57	0.57	0.23	ns
cis-13 18:1	0.09	0.10	0.13	0.03	ns
trans-16 + *cis*-14 18:1	0.23	0.25	0.27	0.09	ns
cis-15 18:1	0.10^{b}	0.11^{b}	0.20^{a}	0.05	**
cis-11 20:1	0.14^{b}	0.18^{a}	0.17^{a}	0.04	†
Other MUFA	0.16^{b}	0.16^{b}	0.23^{a}	0.04	**
Non-conjugated 18:2					
trans-11, *trans*-15 8:2	0.07^{b}	0.09^{b}	0.13^{a}	0.03	**
trans-11, *cis*-15 18:2	0.08^{b}	0.11^{b}	0.37^{a}	0.08	***
18:2 n-6	8.41	8.32	7.82	1.56	ns
Other non-conjugated 18:2	0.55	0.61	0.67	0.15	ns
Conjugated 18:2					
cis-9, *trans*-11 18:2	0.36^{b}	0.70^{b}	1.66^{a}	0.43	***
trans-10, *cis*-12 18:2	0.02^{a}	0.01^{a}	0.02^{b}	0.01	*
Other conjugated 18:2	0.15^{b}	0.18^{b}	0.25^{a}	0.05	**
Other PUFA					
18:3 n-6	0.11	0.13	0.13	0.03	ns
18:3 n-3	0.43^{b}	0.50^{b}	0.96^{a}	0.39	*
20:3 n-6	0.08^{b}	0.07^{b}	0.10^{a}	0.02	*
20:4 n-6	4.49	5.01	4.37	1.42	ns
20:5 n-3	0.44^{b}	0.58^{b}	2.72^{a}	0.85	***
22:4 n-6	0.16	0.19	0.22	0.06	ns
22:5 n-3	1.02^{b}	1.23^{b}	2.21^{a}	0.47	***
22:6 n-3	0.62^{b}	0.72^{b}	1.53^{a}	0.39	***
Other PUFA	0.57^{b}	0.87^{a}	0.77^{ab}	0.28	†
SFA	42.24	38.63	41.63	3.76	ns
MUFA	39.54^{a}	41.32^{a}	33.75^{b}	4.40	**

Table 4: (*Continued*)

	Control	OLI	FO	r.s.d.	*P*-value
PUFA	17.54^{b}	19.31^{b}	23.95^{a}	4.00	*
CLA total	0.48^{c}	0.83^{b}	1.87^{a}	0.43	***
Ratios					
14:1 desaturase index[1]	0.04	0.04	0.04	0.01	ns
18:1 desaturase index[1]	0.74	0.75	0.74	0.03	ns
CLA desaturase index[1]	0.39^{a}	0.37^{a}	0.31^{b}	0.04	**
n-6/n-3	5.44^{a}	5.04^{a}	1.80^{b}	1.50	***

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
$^{a–c}$Means within a row with different superscripts differ significantly.
[1]14:1 desaturase index = *cis*-9 14:1/(14:0 + *cis*-9 14:1); 18:1 desaturase index = *cis*-9 18:1/(18:0 + *cis*-9 18:1); CLA desaturase índex = *cis*-9, *trans*-11 18:2/(*trans*-11 18:1 + cis-9, *trans*-11 18:2).
†$P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 5 *Effect of milk from ewes receiving diets supplemented with Ca soap fatty-acid olive (OLI) and fish (FO) oil on fatty-acid composition (g/100 g of total fatty acid methyl esters) of subcutaneous fat from suckling lambs*

	Control	OLI	FO	r.s.d.	*P*-value
SFA					
6:0	0.04^{b}	0.05^{b}	0.11^{a}	0.03	***
8:0	0.07^{b}	0.07^{b}	0.11^{a}	0.03	**
10:0	0.88	0.89	1.04	0.35	ns
12:0	1.22	1.11	1.19	0.52	ns
14:0	9.00^{b}	10.03^{ab}	11.00^{a}	1.71	*
iso-15:0	0.16^{b}	0.17^{b}	0.21^{a}	0.02	***
anteiso-15:0	0.18^{b}	0.21^{ab}	0.24^{a}	0.05	**
15:0	0.63^{b}	0.72^{ab}	0.84^{a}	0.17	*
iso-16:0	0.19	0.20	0.20	0.03	ns
16:0	31.15^{a}	27.87^{b}	31.91^{a}	2.92	**
17:0	0.92^{b}	0.96^{b}	1.21^{a}	0.17	***
18:0	11.81^{a}	11.67^{a}	8.38^{b}	1.91	***
20:0	0.11^{ab}	0.13^{a}	0.10^{b}	0.03	†
Other SFA	0.37^{b}	0.37^{b}	0.47^{a}	0.05	***
MUFA					
cis-9 10:1	0.06^{b}	0.07^{b}	0.09^{a}	0.03	†
cis-9 14:1	0.25^{b}	0.32^{a}	0.35^{a}	0.06	**
trans-9 16:1 + iso-17:0	0.35^{b}	0.38^{b}	0.65^{a}	0.07	***
cis-7 16:1	0.38^{b}	0.35^{b}	0.45^{a}	0.05	***
cis-9 16:1 + anteiso-17:0	2.40^{b}	2.44^{b}	3.35^{a}	0.41	***
cis-13 16:1	0.10^{b}	0.13^{b}	0.17^{a}	0.06	**
cis-9 17:1	0.46^{b}	0.47^{b}	0.59^{a}	0.12	*
trans-6 + *trans*-7 + *trans*-8 18:1	0.29^{b}	0.68^{a}	0.22^{b}	0.10	***
trans-9 18:1	0.29^{c}	0.62^{a}	0.36^{b}	0.09	***
trans-10 18:1	0.37^{b}	1.11^{a}	0.61^{b}	0.69	*
trans-11 18:1	0.79^{c}	1.97^{b}	5.44^{a}	0.94	***
trans-12 18:1	0.20^{b}	0.48^{a}	0.43^{a}	0.13	***
cis-9 18:1	32.74^{a}	31.25^{a}	23.98^{b}	4.47	***
trans-15 + *cis*-11 18:1	0.52^{c}	0.66^{b}	0.98^{a}	0.13	***
cis-12 18:1	0.23^{a}	0.27^{a}	0.17^{b}	0.08	*
cis-13 18:1	0.06^{b}	0.09^{a}	0.09^{a}	0.02	***
trans-16 + *cis*-14 18:1	0.27^{b}	0.36^{a}	0.19^{c}	0.10	**
cis-15 18:1	0.07^{c}	0.10^{b}	0.14^{a}	0.04	***
cis-11 20:1	0.08^{c}	0.11^{b}	0.17^{a}	0.05	***
Non-conjugated 18:2					
trans-11, *cis*-15 18:2	0.04^{b}	0.17^{b}	0.51^{a}	0.22	***
18:2 n-6	1.48^{a}	1.02^{b}	0.93^{b}	0.31	***
Other non-conjugated 18:2	0.51^{b}	0.71^{a}	0.53^{b}	0.13	**

Table 5: (*Continued*)

	Control	OLI	FO	r.s.d.	*P*-value
Conjugated 18:2					
cis-9, *trans*-11 18:2	0.18[b]	0.37[a]	0.37[a]	0.20	*
trans-10, *cis*-12 18:2	0.00[c]	0.01[b]	0.01[a]	0.00	***
Other conjugated 18:2	0.06[c]	0.08[b]	0.10[a]	0.02	***
Other PUFA					
18:3 n-6	0.02	0.03	0.02	0.02	ns
18:3 n-3	0.13	0.14	0.15	0.05	ns
20:4 n-6	0.04	0.04	0.02	0.02	ns
20:5 n-3	0.02	0.02	0.03	0.03	ns
22:5 n-3	0.03	0.03	0.04	0.02	ns
22:6 n-3	0.02[a]	0.02[a]	0.02[b]	0.01	*
Other PUFA	0.04[c]	0.06[b]	0.12[c]	0.02	***
SFA	56.87	54.57	57.26	3.93	ns
MUFA	39.96	41.96	38.58	3.86	ns
PUFA	2.55	2.66	2.86	0.55	ns
CLA total	0.24[b]	0.45[a]	0.48[a]	0.20	*
Ratios					
14:1 desaturase index[1]	0.03	0.03	0.03	0.00	ns
18:1 desaturase index[1]	0.75[b]	0.76[b]	0.80[a]	0.03	***
CLA desaturase index[1]	0.18[a]	0.19[a]	0.07[b]	0.09	**
n-6/n-3	8.32[a]	5.56[b]	4.33[c]	1.28	***

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
[a–c]Means within a row with different superscripts differ significantly.
[1]14:1 desaturase index = *cis*-9 14:1/(14:0 + *cis*-9 14:1); 18:1 desaturase index = *cis*-9 18:1/(18:0 + *cis*-9 18:1); CLA desaturase índex = *cis*-9, *trans*-11 18:2/(*trans*-11 18:1 + *cis*-9, *trans*-11 18:2).
†$P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

with < 14 carbon atoms characteristic of body fat. These differences could be attributed to the fact that the suckling lambs were fed exclusively on maternal milk until their slaughter, as well as to their digestive physiology, which is similar to monogastrics where the rumen is not functional, and hence no BH of the milk FAs takes place (Osorio *et al*., 2007; Scerra *et al*., 2007; Manso *et al*., 2011). Thus, OLI treatment resulted in a significant percentage decrease in 16:0 (Figures 2 and 3) and an increase in most *trans*-18:1 in intramuscular and subcutaneous fats ($P < 0.05$). As in milk fat, the greatest differences in intramuscular fat were observed in the FA profile of FO treatment. In this respect, the observed decreases in 18:0 and *cis*-9 18:1 content as well as the increases in VA (Figure 2) are noteworthy; similarly, the RA content in both fat depots ($P < 0.05$) and n-3 PUFA in intramuscular fat ($P < 0.001$).

Both fat depots presented differences in their FA profiles. Overall, intramuscular fat had a higher percentage of PUFAs together with a lower proportion of SFAs (Tables 4 and 5). Strong coefficients of correlation were established for 20:5 n-3 ($r = 0.86$; $P < 0.001$), 22:5 n-3 ($r = 0.77$; $P < 0.001$), 22:6 n-3 ($r = 0.77$; $P < 0.001$), RA ($r = 0.81$; $P < 0.001$) and *trans*-11 18:1 ($r = 0.86$; $P < 0.001$) between dam milk and suckling lamb intramuscular fats. Suckling lambs preferentially incorporate PUFAs into muscle rather than storing them in the adipose tissue because of their important metabolic roles. The major presence of PUFAs in intramuscular fat is because of the higher proportion of phospholipids in these depots. Phospholipids are essential constituents of cell membranes and require high levels of PUFAs to maintain membrane properties and physiological functions. Ashes *et al*. (1992) showed that neither 20:5 n-3 and 22:6 n-3 were incorporated into the triacylglycerides of the muscle and adipose tissue, but were transferred into the phospholipids in cattle fed with protected supplements of FO.

The intramuscular fat depots obtained from the FO suckling lambs had the highest levels of VA and RA, and a significant positive correlation was observed between both FAs in this tissue ($r = 0.98$, $P < 0.001$). This result was probably because of the dual origin of *cis-9*, *trans*-11 18:2 derived both from the diet and by endogenous synthesis from *trans*-11 18:1. The fact that *cis-9*, *trans*-11 18:2 is less abundant in the subcutaneous depot could be partly explained by the greater Δ-9 desaturase activity in the intramuscular tissue (Tables 4 and 5). These results coincide with those of Palmquist *et al*. (2004) who reported a higher endogenous synthesis of *cis-9*, *trans*-11 18:2 from *trans*-11 18:1 in the muscle than in the lamb adipose tissue.

Despite being more abundant in Control than in OLI and FO milk, linoleic acid contents were found to be similar ($P > 0.05$) in carcass intramuscular deposits in all treatments (Table 4). These results indicate that diet influenced the muscle content of n-3 FAs more than that of 18:2 n-6, in accordance with previous observations in lamb (Palmquist *et al*., 2004). Furthermore, Wood *et al*. (2008) reported that in meat fat, linoleic-acid incorporation in relation to the

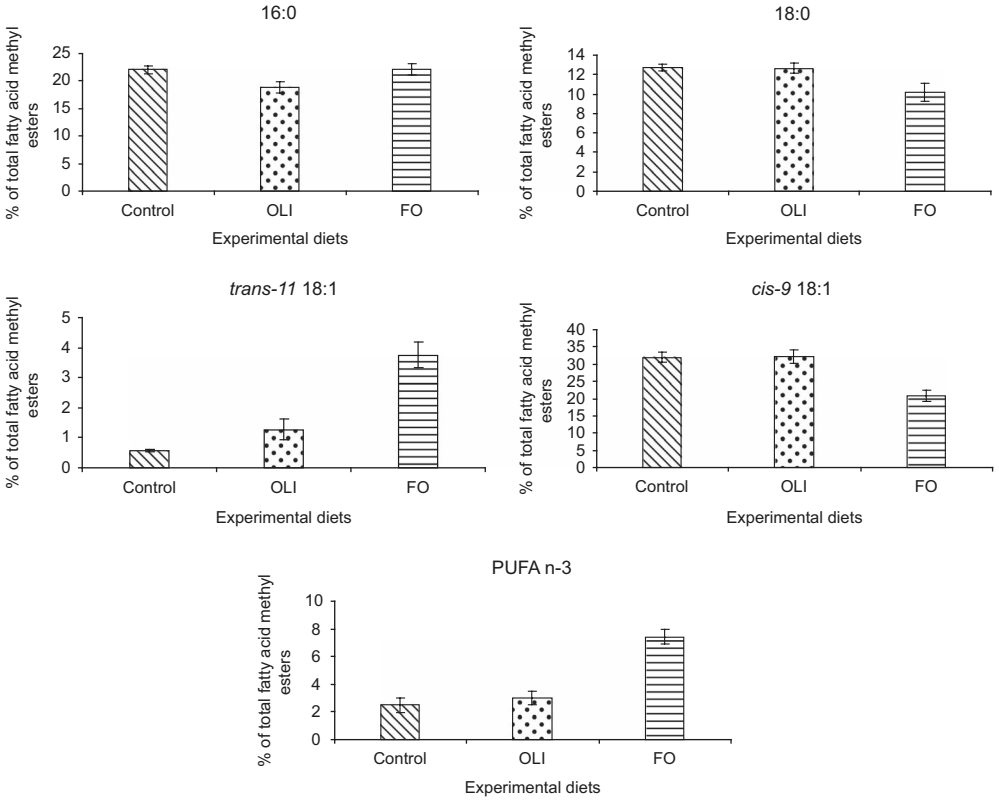

Figure 2 Mean values of 16:0, 18:0, *cis*-9 18:1, *trans*-11 18:1 and total n-3 fatty acids (18:3 + 20:5 + 22:5 + 22:6) of intramuscular fat from suckling lambs nourished with ewes milk fed three experimental diets (forage/concentrate ratio 50 : 50), each supplemented with either 3% Ca soap fatty acids of palm (Control), olive (OLI) or fish (FO) oil.

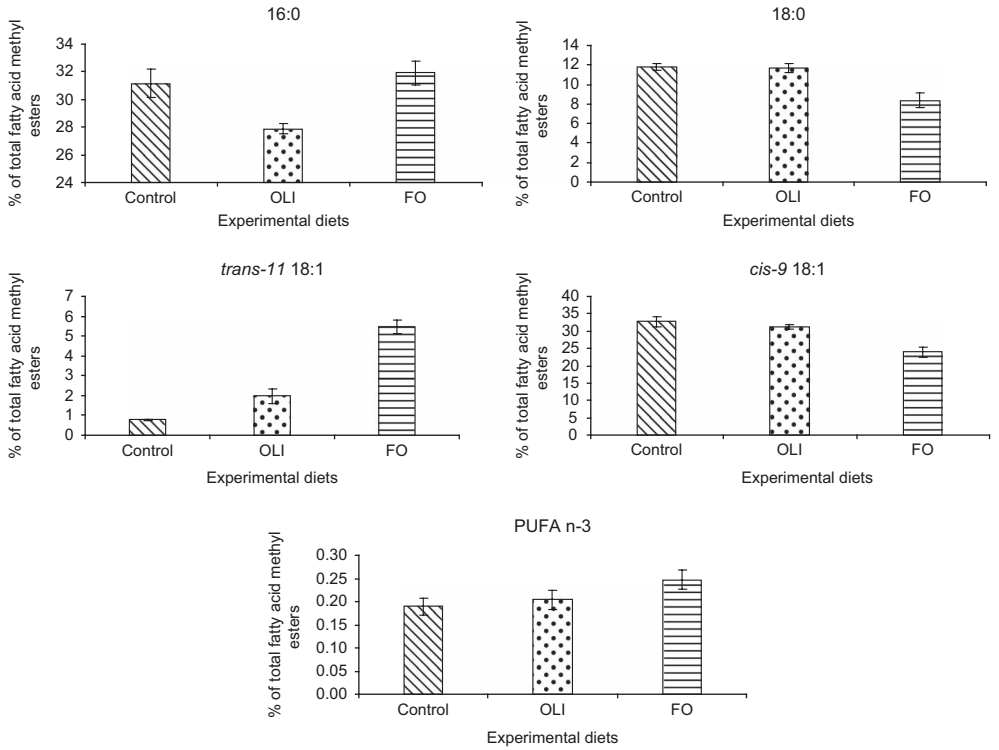

Figure 3 Mean values of 16:0, 18:0, *cis*-9 18:1, *trans*-11 18:1 and total n-3 fatty acids (18:3 + 20:5 + 22:5 + 22:6) of subcutaneous fat from suckling lambs nourished with ewes milk fed three experimental diets (forage/concentrate ratio 50:50), each supplemented with either 3% Ca soap fatty acids of palm (Control), olive (OLI) or fish (FO) oil.

amount in the diet is greater than for other FAs. However, despite the increase in n-6 FAs, the n-6/n-3 ratio decreased three fold with the FO diet ($P < 0.001$) compared with Control and OLI diets. This value in intramuscular fat (Table 4) is clearly below 4, which would make this fat highly recommendable for consumption (Simopoulos, 2008). Intramuscular fat is irreversibly connected with meat and it cannot be removed before human consumption, unlike visible fat, such as subcutaneous fat. In addition, given the higher PUFA and CLA composition and the lower SFA content of intramuscular fat compared with removable depot fat, the relevance of intramuscular fat for the intake of PUFAs and other potential FAs may be greater than expected at first sight.

Conclusions

The supplementation of ewe diets with different CSFAs did modify the FA profile of milk fat. CSFAs of FO produced more important changes than the supplementation of the ewe diet with CSFAs of OLI. While in the assayed conditions the addition of CSFAs of FO decreased the milk fat content, it also significantly increased healthy FAs, such as n-3 PUFAs and RA. Moreover, this took place without a simultaneous increase in either SFAs or *trans*-FAs such as *trans*-10 18:1, with potentially negative effects on consumers' health. This milk FA profile was reflected in the intramuscular and subcutaneous fat of suckling lambs, which would make this nutritional strategy adequate for producing ruminant products with a healthier FA profile.

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References

AbuGhazaleh AA and Jenkins TC 2004. Docosahexaenoic acid promotes vaccenic acid accumulation in mixed rumen cultures when incubated with linoleic acid. Journal of Dairy Science 87, 1047–1050.

Ashes JR, Sieber BD, Gulati SK, Cuthberson AZ and Scott TW 1992. Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants. Lipids 27, 629–631.

Association of Official Analytical Chemists 2003. Official method of analysis of the Association of Official Agricultural Chemists, 17th edition. AOAC International, Gaithersburg, MD, USA.

Awawdeh MS, Obeidat BS and Kridli RT 2009. Yellow grease as an alternative energy source for nursing Awassi ewes and their suckling lambs. Animal Feed Science and Technology 152, 165–174.

Bichi E, Toral PG, Hervás G, Frutos P, Gómez-Cortés P, Juárez M and De la Fuente MA 2012. Inhibition of Δ9-desaturase activity with sterculic acid: effect on the endogenous synthesis of cis-9 18:1 and cis-9 trans-11 18:2 in dairy sheep. Journal of Dairy Science 95, 5242–5252.

Bligh EG and Dyer WJ 1959. A rapid method for total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Casals R, Caja G, Pol MV, Such X, Albanell E, Gargouri G and Casellas J 2006. Response of lactating dairy ewes to various levels of dietary calcium soaps of fatty acids. Animal Feed Science and Technology 131, 312–332.

Doreau M, Fievez V, Troegeler-Meynadier A and Glasser F 2012. Métabolisme ruminal et digestion des acides gras longs chez le ruminant: le point des connaissances récentes. INRA Productions Animales 25, 361–374.

Gama MAS, Garnsworthy PC, Griinari JM, Leme PR, Rodrigues PHM, Souza LWO and Lanna DPD 2008. Diet-induced milk fat depression: association with changes in milk fatty acid composition and fluidity of milk fat. Livestock Science 115, 319–331.

Gargouri A, Caja G, Casals R and Mezghan I 2006. Lactational evaluation of effects of calcium soap of fatty acids on dairy ewes. Small Ruminant Research 66, 1–10.

Gómez-Cortés P, Frutos P, Mantecón AR, Juárez M, De la Fuente MA and Hervás G 2008. Addition of olive oil to dairy ewe diets: effect on milk fatty acid profile and animal performance. Journal of Dairy Science 91, 3119–3127.

Gulati SK, Ashes JR and Scott TW 1999. Hydrogenation of eicosapentaenoic and docosahexaenoic acids and their incorporation into milk fat. Animal Feed Science and Technology 79, 57–64.

Hussein M, Harvatine KH, Weerasinghe PB, Sinclair LA and Bauman DE 2013. Conjugated linoleic acid-induced milk fat depression lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. Journal of Dairy Science 96, 3825–3834.

International Dairy Federation 2000. International IDF Standard 141C:2000. Determination of milk fat, protein and lactose content. Guidance on the operation of mid-infrared instruments. International Dairy Federation, Brussels, Belgium.

Jenkins TC and Bridges WC Jr. 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. European Journal of Lipid Science and Technology 109, 778–789.

Kitessa SM, Peake D, Bencini R and Williams AJ 2003. Fish oil metabolism in ruminants: III. Transfer of n-3 polyunsaturated fatty acids (PUFA) from tuna oil into sheep's milk. Animal Feed Science and Technology 108, 1–14.

Kitessa SM, Gulati SK, Ashes JR, Fleck E, Scott TW and Nichols PD 2001. Utilisation of fish oil in ruminants – I. Fish oil metabolism in sheep. Animal Feed Science and Technology 89, 189–199.

Luna P, Bach A, Juárez M and De la Fuente MA 2008. Effect of a diet enriched in whole linseed and sunflower oil on goat milk fatty acid composition and conjugated linoleic acid isomer profile. Journal of Dairy Science 91, 20–28.

Manso T, Bodas R, Vieira C, Mantecón AR and Castro T 2011. Feeding vegetable oils to lactating ewes modifies the fatty acid profile of suckling lambs. Animal 5, 1659–1667.

Mosley EE, Powell GL, Riley MB and Jenkins TC 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. Journal of Lipid Research 43, 290–296.

Or-Rashid MM, Kramer JKG, Wood MA and McBride BW 2008. Supplemental algal meal alters the ruminal *trans*-18:1 fatty acid and conjugated linoleic acid composition in cattle. Journal of Animal Science 86, 187–196.

Osorio MT, Zumalacárregui JM, Figueira A and Mateo J 2007. Fatty acid composition in subcutaneous, intermuscular and intramuscular fat deposits of suckling lamb meat: effect of milk source. Small Ruminant Research 73, 127–134.

Palmquist DL, St-Pierre N and McClure KE 2004. Tissue fatty acid profiles can be used to quantify endogenous rumenic acid synthesis in lambs. Journal of Nutrition 134, 2407–2414.

Pérez Alba LM, De Souza Cavalcanti S, Pérez Hernández M, Martínez Marín A and Fernández Marín G 1996. Calcium soap of olive fatty acids in the diets of Manchega dairy ewes: effects on digestibility and production. Journal of Dairy Science 80, 3316–3324.

Perfield JW, Lock AL, Griinari JM, Sæbø A, Delmonte P, Dwyer DA and Bauman DE 2007. Trans-9, cis-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. Journal of Dairy Science 90, 2211–2218.

Pulina G, Nudda A, Battacone G and Cannas A 2006. Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk. Animal Feed Science and Technology 131, 255–291.

Scerra M, Caparra P, Foti F, Galofaro V, Sinatra MC and Scerra V 2007. Influence of ewe feeding systems on fatty acid composition of suckling lambs. Meat Science 76, 390–394.

Shingfield KJ, Bonnet M and Scollan ND 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7, 132–162.

Shingfield KJ, Sæbø A, Sæbø PC, Toivonen V and Griinari JM 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. Journal of Dairy Science 92, 4317–4329.

Shingfield KJ, Reynolds CK, Hervás G, Griinari JM, Grandison AS and Beever DE 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. Journal of Dairy Science 89, 714–732.

Simopoulos AP 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Experimental Biology and Medicine 233, 674–688.

Sinclair LA, Lock AL, Early R and Bauman DE 2007. Effects of trans-10, cis-12 conjugated linoleic acid on ovine milk fat synthesis and cheese properties. Journal of Dairy Science 90, 3326–3335.

Toral PG, Frutos P, Hervás G, Gómez-Cortés P, Juárez M and De la Fuente MA 2010a. Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. Journal of Dairy Science 93, 1604–1615.

Toral PG, Hervás G, Gómez-Cortés P, Frutos P, Juárez M and De la Fuente MA 2010b. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. Journal of Dairy Science 93, 1655–1667.

Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI and Wittington FM 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Science 78, 343–358.